Vol. 101. N.º 6, pp. 408-412, 2009

Alteration of the renal regulatory hormonal pattern during experimental obstructive jaundice

F. J. Padillo[1*], A. Cruz[1*], I. Espejo[2], M. Barcos[2], M. Gómez-Álvarez[1] and J. Muntané[3*]

[1]Departments of General Surgery, [2]Clinical Analysis and [3]Research Unit. Reina Sofía University Hospital. Córdoba, Spain. [*]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd)

ABSTRACT

Objective: the alteration of hormones regulating sodium and water status is related to renal failure in obstructive jaundice (OJ).

Experimental design: OJ was induced by common bile duct ligation. Samples were obtained from the control (SO) and OJ groups at 24 and 72 hours, and at 7 days. Different parameters related to biliary obstruction, liver and renal injury, and vasoactive mediators such as renin, aldosterone, endothelin-1 (ET-1) and prostaglandin E_2 (PGE_2) were studied.

Results: bile duct ligation caused an increase in total bilirubin ($p < 0.001$) and alkaline phosphatase (AP) ($p < 0.001$). The SO and OJ groups had the same values for diuresis, renin, and creatinine clearance at 24 h. However, animals with OJ had a lower sodium concentration in urine than SO animals ($p < 0.01$), as well as an increase in aldosterone levels ($p < 0.03$). ANP levels were moderately increased during OJ but did not reach statistical significance when compared to the SO group. In contrast, OJ animals showed a rise in serum ET-1 concentration ($p < 0.001$) and increased PGE_2 in urine ($p < 0.001$).

Conclusions: biliary obstruction induced an increase in ET-1 release and PGE_2 urine excretion. These hormones might play a role during the renal complications associated with renal disturbances that occur during OJ.

Key words: Aldosterone. Creatinine. Endothelin-1. Obstructive jaundice. Renal dysfunction. Renin.

Padillo FJ, Cruz A, Espejo I, Barcos M, Gómez-Álvarez M, Muntané J. Alteration of the renal regulatory hormonal pattern during experimental obstructive jaundice. Rev Esp Enferm Dig 2009; 101: 408-412.

Received: 03-03-09.
Accepted: 12-03-09.

Correspondence: F. J. Padillo. Professor of Surgery. Unidad de Cirugía y Trasplante Pancreático. Hospital Universitario Reina Sofía. Avda. Menéndez Pidal, s/n. 14004 Córdoba, Spain. e-mail: javierpadiruiz@hotmail.com

ABBREVIATIONS

AP: alkaline phosphatase.
ET-1: endothelin-1.
OJ: obstructive jaundice.
PGE_2: prostaglandin E_2.
SO: Sham operated.

INTRODUCTION

Cholestasis is a clinical and biochemical syndrome caused by the impaired bile flow often associated with extrahepatic complications (1). Acute renal failure is a major complication of obstructive jaundice (OJ), with 8% incidence in several series (2). Biliary obstruction was first linked to renal failure in the early study carried out by Clairmont and von Haberer (1910) (3). Several hypotheses have sought to explain the pathogenesis of renal dysfunction in OJ. The depletion of the extracellular compartment (4), myocardial dysfunction, and altered hemodynamic status (5) have been linked to renal failure in biliary obstruction. In addition, severe oxidative stress has been implicated in the renal dysfunction associated to experimental OJ (6,7) as well as other liver diseases (8). Other lines of research have looked at the involvement of vasoactive mediators such as endothelin-1 (ET-1). ET-1 is a powerful paracrine vasoconstrictor that acts by means of specific type-A and type-B receptors. In biochemical terms, it is a 21-amino acid peptide derived from what is known as big ET-1 by the action of the endothelin-converter enzyme. It is synthesized mainly by endothelial cells and to a lesser degree by stellate cells in the liver and biliary epithelium (9,10). ET-1 changes have been related to different human diseases (11). ET-1 both acts on the mesangial cells and causes vasoconstriction in the renal microcirculation, which may contribute – along-

side factors such as endotoxemia, extracellular volume depletion, and myocardial dysfunction, to a drop in glomerular filtration and the development of renal failure in OJ. However, vasoactive compensatory hormones such as prostaglandin E_2 (PGE_2) and atrial natriuretic peptide (ANP) may play a role in the preservation of renal function during OJ (5,6). The aim of this study was to determine the status of hormones regulating sodium and water, as well as different vasoactive mediators affecting renal function during experimental OJ.

METHODS

Animals

Male Wistar rats (300-375 g) were subjected to controlled conditions of temperature (about 22 ºC) and illumination (12 h light:12 h dark cycle), and were provided with food (Ebro Agrícolas®, Seville, Spain) and water *ad libitum*. Food metabolized energy at 2375 cal/kg. Rats were put in metabolic cages for several days before surgery, and for seven days after surgery. The animals were treated according to institutional guidelines and the study was approved by the Research Committee of Reina Sofia University Hospital.

Experimental design

Animals were divided into three groups: baseline (n = 5), sham operated (SO, n = 15), and OJ (n = 15) for 7 days. The SO and OJ groups were divided into three subgroups for extracting samples at 24 and 72 hours, as well as at 7 days after surgery. Animals were anesthetized with pentobarbital (Nembutal® 50 mg/kg/i.p.) for all surgical procedures. Cephazoline (17 mg/kg/i.m.) was used as antibiotic prophylaxis. SO animals were submitted to laparotomy and abdominal closure without bile duct intervention. The procedure for OJ was initiated by a midline ventral incision with exposure of the extra-hepatic bile duct. A double ligature with silk suture was tied and the bile duct was sectioned. A two-layer running suture was used for abdominal closure with Dexon (Braun Dexon, Barcelona, Spain) and Mersilk (Ethicon, Brussels, Belgium) material. The animals were killed under anesthesia 24 hours, 72 hours or 7 days later. Blood was collected from the abdominal aorta and the serum was frozen at -20 ºC until biochemical parameters were measured. For hormone assays and ET-1, blood samples were collected in chilled tubes containing EDTA (2 mg/ml) and aprotinin. Samples were centrifuged at 3000 rpm for 10 min at 4 ºC, within 30 min of collection. The plasma was decanted and stored frozen at -70 ºC for subsequent analysis. Urine was collected in plastic tubes, centrifuged at 3500 rpm for 20 min at 4 ºC, aliquoted, and frozen at -20 ºC for later study.

Determination of biochemical parameters

Total bilirubin, alkaline phosphatase (AP), sodium, and creatinine were measured in the serum using an Axon autoanalyzer (Bayer Diagnostics, Tarrytown, NY, USA). Sodium and creatinine were also measured in the urine using an Axon autoanalyzer (Bayer Diagnostics, Tarrytown, NY, USA). Creatinine clearance was calculated using serum creatinine and 24-hour urinary creatinine excretion.

Measurement of ET-1, PGE_2, and hormones

Plasma ET-1 levels were determined by enzyme immunoassay (kit BBE5) according to the manufacturer's instructions (RD Systems Europe, Abingdon, UK). PGE_2 in urine was determined by enzyme immunoassay (kit DE0100) according to the manufacturer's instructions (RD Systems Europe, Abingdon, UK). The amount of PGE_2 excreted in urine was calculated from the formula: concentration of PGE_2 in urine x volume per minute of urine.

ANP, renin, and aldosterone

Radioimmunoassay techniques with intra-assay variation coefficients of 5 and 6% were used to determine ANP (h-ANP, Cob. I-AR55TM Tokyo, Japan; reference values 20-60 pg/ml), renin (a-Renin IRMA 4K-REN-00TM; Future Diagnostics, Wijchen, The Netherlands), and aldosterone (ACTIVETM aldosterone; Diagnostic Systems Laboratories, Webster, Texas, USA) levels.

Statistical analysis

A multivariate ANOVA model, known as the General Linear Model for repeated measurements, was used. A significance level of $p < 0.1$ was allowed for this model. To compare quantitative variables, Student's t test was used for non-paired data. The SPSS 7.5 programme for Windows conducted the statistical analysis.

RESULTS

The mean values of the parameters studied are found in table I. Animals with bile duct obstruction showed an increase in cholestasis, total bilirubin, and AP when compared to SO animals. The evaluation of renal function parameters (24-hour diuresis and creatinine clearance) in animals with OJ showed a trend towards more diuresis *versus* SO animals, though differences were not significant. In addition, animals with OJ had a lower sodium concentration in urine as compared to its increase throughout the study in SO animals. Hormones in the

Table I. Comparison of variables in rats with obstructive jaundice (OJ) *vs.* sham operation (SO)

	Baseline	*24 h*	*72 h*	*7 days*	*p intragroup*	*Intergroup*
Total bilirubin (mg/dl)						
SO	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	n.s.	< 0.001
OJ	0.1 ± 0.01	3.05 ± 0.300	7.2 ± 1.90	7.2 ± 2.70	< 0.001	
Alkaline phosphatase (U/l)						
SO	240 ± 90	271 ± 46	209 ± 43	205 ± 45	n.s	< 0.001
OJ	236 ± 95	1925 ± 303	870 ± 148	660 ± 299	< 0.001	
Urine output (ml/24 h)						
SO	10 ± 2.9	8.8 ± 4.50	10 ± 2.7	9.8 ± 1.00	n.s.	n.s.
OJ	13 ± 4.2	13 ± 6.6	11 ± 3.2	12 ± 5.1	n.s.	
Creatinine clearance (ml/min)						
SO	1.68 ± 1.070	1.33 ± 0.760	1.31 ± 0.280	1.11 ± 0.270	n.s.	n.s.
OJ	1.71 ± 1.060	0.93 ± 0.38	1.45 ± 1.27	1.38 ± 0.39	n.s	
Excretion sodium fraction						
SO	0.53 ± 0.100	0.24 ± 0.200	0.56 ± 0.200	0.75 ± 0.100	< 0.01	n.s.
OJ	0.53 ± 0.200	0.34 ± 0.100	0.46 ± 0.200	0.36 ± 0.100	n.s	
Urine sodium (mmol/l)						
SO	147 ± 18	62 ± 33	140 ± 62	171 ± 41	< 0.002	< 0.01
OJ	145 ± 17	53 ± 21	84 ± 30	99 ± 55	< 0.001	
Renin (µU/ml)						
SO	1.11 ± 1.610	3.62 ± 3.620	1.14 ± 1.540	0.43 ± 0.950	n.s.	n.s.
OJ	1.11 ± 1.520	1.65 ± 3.170	1.40 ± 2.980	2.32 ± 3.270	n.s.	
Aldosterone (pg/ml)						
SO	561 ± 489	593 ± 266	378 ± 242	302 ± 80	n.s.	< 0.03
OJ	561 ± 461	931 ± 381	521 ± 315	624 ± 381	n.s.	
ANP (pg/100 µl)						
SO	1.88 ± 0.850	2.27 ± 0.630	1.57 ± 0.440	1.76 ± 0.740	n.s.	n.s.
OJ	1.84 ± 0.840	1.42 ± 0.530	1.84 ± 0.950	1.95 ± 0.910	n.s.	
Endothelin (fmol/ml)						
SO	0.34 ± 0.010	0.43 ± 0.110	0.36 ± 0.010	0.35 ± 0.010	n.s.	< 0.001
OJ	0.34 ± 0.010	0.82 ± 0.230	0.51 ± 0.120	0.60 ± 0.270	< 0.001	
Urine PGE_2 (ng/l)						
SO	7.56 ± 2.450	15.5 ± 4.40	19.69 ± 13.540	18.17 ± 8.450	< 0.055	< 0.001
OJ	7.56 ± 2.310	26 ± 15.60	47 ± 8.7	41 ± 14.0	< 0.001	
Urine PGE_2 excreted amount (pg/min)	53 ± 12	85 ± 23	134 ± 11	123 ± 60	n.s.	< 0.001
	53 ± 13	226 ± 131	356 ± 91	362 ± 168	< 0.001	

renin-angiotensin-aldosterone axis were determined. There were no significant differences between the SO and OJ groups for serum renin concentrations. However, serum aldosterone in OJ rats was two-fold that of SO rats. There was a rise in ANP in animals with OJ, although there were no significant differences with the SO group. ET-1 and PGE_2 in urine were significantly enhanced in OJ animals as compared to SO animals. SO animals also showed a significant increase in PGE_2 throughout the study. When ET-1 and PGE_2 were compared with creatinine clearance in animals with OJ, rats with poorer renal function had higher ET-1 values and lower PGE_2 concentrations in urine (Figs. 1 and 2).

DISCUSSION

One of the main consequences of biliary obstruction is its effect on renal function, which markedly increases patient morbidity and mortality. The experimental study reported here tried to determine whether certain factors that affect renal perfusion contributed to the renal failure occurring in OJ. Although the rise in total bilirubin and AP showed that bile duct ligation induced cholestasis, the data related to renal function such as 24-hour diuresis and/or creatinine clearance showed no significant differences between the OJ and SO groups, though diuresis was higher in OJ rats. This could be attributed to the short

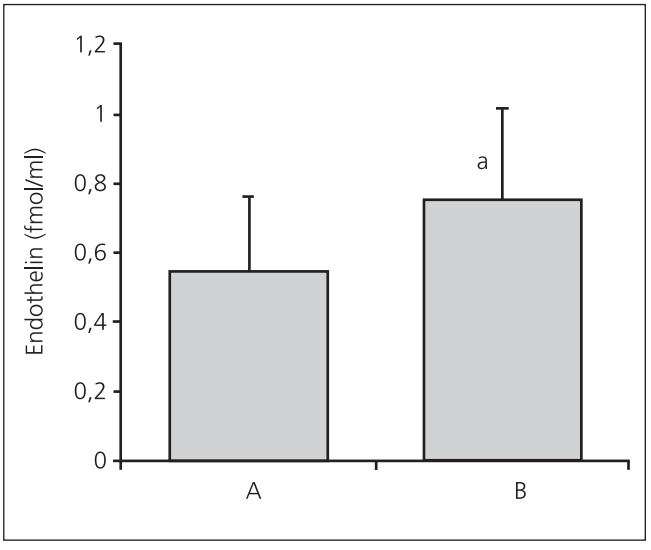

Fig. 1. Serum endothelin in jaundiced rats with creatinine clearance over (A) and under (B) mean values. a: $p < 0.02$.

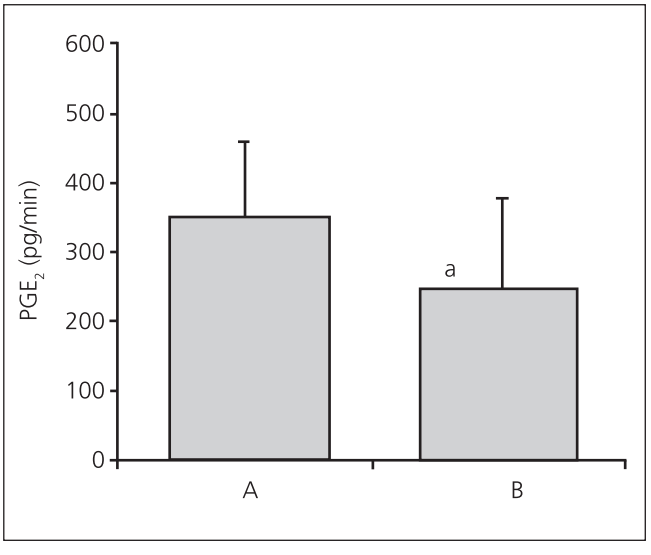

Fig. 2. Urine PGE_2 excreted in jaundiced rats with creatinine clearance over (A) and under (B) mean values. a: $p < 0.03$.

period of OJ. However, studies with similar experimental models showed a significant reduction in creatinine clearance at four days of biliary ligation (11,12), and a rise in serum creatinine in animals submitted to one week of OJ (7,13). Our study corroborated previous studies (14,25) that showed that OJ causes a decrease in sodium excretion and lower sodium concentrations in urine, which suggests increased sodium reabsorption in cholestatic rats. In addition, our study could not show a clear relationship between sodium excretion or sodium concentration in urine and ANP concentration in urine. In a prospective study of patients with OJ, our group showed that obstruction of the biliary tree is linked to a rise in ANP concentration in plasma (15). The rise in ANP concentration in the blood from OJ animals did not reach statistical differences when compared to the SO group. Nevertheless, it could be feasible that the tendency of ANP to rise could be statistically significant at the longest time period of OJ. Another factor to bear in mind is that our experiment was conducted on rats, and studies with a rise on ANP have been published in humans (15) and in rabbits (14). Nonetheless, the concentration of renin and aldosterone in the serum increased in rats with biliary obstruction, though only aldosterone levels were statistically significant. The effects of aldosterone are sodium retention and potassium excretion in the distal tubule. Our study found a drop in sodium excretion, and the concentration of sodium in urine that was related to the increase in aldosterone concentration, which suggests a rise in sodium retention in the distal tubule. In this respect, results corroborate clinical studies run by our group, which showed that patients with OJ suffered a depletion of extracellular volume that is directly related to biliary obstruction (16), and had renin and aldosterone levels in the upper range of normality (5).

ET-1 is a peptide with a powerful vasoconstrictor action that is involved in the genesis of several diseases, mainly in the hepato-biliary area. Therefore, it has been suggested that ET-1 has a key function in serious complications of hepatic cirrhosis such as portal hypertension (17) and hepatopulmonary syndrome (18). It was recently shown that the blocking of ET-1 receptors improved microcirculation and sinusoidal perfusion in an experimental model of acute liver failure (19). Our findings indicated that in experimental OJ there was a rise in plasma ET-1 concentrations, which reached its peak 24 hours after the induction of biliary obstruction. However, other authors have demonstrated that the highest values of ET-1 occur four to five weeks after biliary ligation associated with biliary cirrhosis (20,21). Although in our study renal function parameters showed no differences between the OJ and SO groups, the concentration of ET-1 was higher in rats with creatinine clearance below the mean value, which suggests that rats with higher ET-1 are at greater risk of renal failure. Nevertheless, other experimental models of OJ have reported renal failure (12,13). In addition, these studies found increased ET-1 synthesis in the renal papilla, which may contribute to renal dysfunction and predispose to acute renal failure during OJ (12). A clinical study of pediatric patients with cirrhosis showed a negative correlation between plasma ET-1 and creatinine clearance (22), which corroborates the link between renal dysfunction and OJ. In addition, it has been shown that ET-1 induces renal synthesis of PGE_2, which exerts a vasodilatory effect on renal vascular tone (23). Our study showed increased PGE_2 urine secretion in OJ rats. Moreover, rats with creatinine clearance below the mean value had less PGE_2 content excreted to urine. This suggests that PGE_2 could exert a protective role in kidneys against renal function deterioration. Along the same

lines, other authors have shown a rise in PGE_2 in the urine of rats with OJ (24). In addition, clinical studies signaled a normalization of PGE_2 in urine after biliary drainage in patients with OJ (25).

In summary, our study shows that during the first week after bile duct ligation an increase in plasma ET-1 concentrations and a rise in PGE_2 secretion to urine were seen. The stable renal function found in this period might be related to a compensatory mechanism between hormones regulating sodium and water, as well as vasoactive hormones.

ACKNOWLEDGEMENTS

This study was supported by Programa de Promoción de la Investigación en Salud del Ministerio de Sanidad y Consumo (FIS 02/0155, RTIC C03/03, G03/156). CIBERehd is funded by Instituto de Salud Carlos III.

REFERENCES

1. Pérez-Fernández T, López-Serrano P, Tomás E, Gutiérrez ML, Lledó JL, Cacho G, et al. Diagnostic and therapeutic approach to cholestatic liver disease. Rev Esp Enferm Dig 2004; 96: 60-73.
2. Fogarty BJ, Parks RW, Rowlands BJ, Diamond T. Renal dysfunction in obstructive jaundice. Br J Surg 1995; 82: 877-84.
3. Clairmont P, von Haberer H. Über anurie nach gallensteinoperationen. Mitt Grenzgeb Med Chir 1910; 22: 159-72.
4. Padillo FJ, Briceño J, Cruz A, Chicano M, Naranjo A, Vallejo J, et al. Randomized clinical trial of the effect of intravenous fluid administration in patients with obstructive jaundice undergoing endoscopic drainage. Br J Surg 2005; 92: 39-43.
5. Padillo FJ, Puente J, Gómez M, Dios F, Naranjo A, Vallejo JA, et al. Improved cardiac function in patients with obstructive jaundice after internal biliary drainage: hemodynamic and hormonal assessment. Ann Surg 2001; 234: 652-6.
6. Cruz A, Padillo FJ, Tunez I, Muñoz C, Granados J, Pera-Madrazo C, et al. Melatonin protects against renal oxidative stress after obstructive jaundice in rats. Eur J Pharmacol 2001; 425: 135-9.
7. Kucuk C, Sozuer E, Ikizceli I, Avsarogullari L, Keceli M, Akgun H, et al. Role of oxygen free radical scavengers in acute renal failure complicating obstructive jaundice. Eur Surg Res 2003; 35: 143-7.
8. Conde de la Rosa L, Moshage H, Nieto N. Hepatocyte oxidant stress and alcoholic liver disease. Rev Esp Enferm Dig 2008; 100: 156-63.
9. Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, de Wit D, et al. A membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. Cell 1994; 78: 473-85.
10. Pinzani M, Milani S, DeFranco R, Grappone C, Caligiuri A, Gentilini A, et al. Endothelin 1 is overexpressed in human cirrhotic liver and exerts multiple effects on activated hepatic stellate cells. Gastroenterology 1996; 110: 534-48.
11. Ferrero-Herrero E, García-Villalón AL, Labalde-Martínez M, Diéguez-Castrillo G. Effect of endothelin-1 on tumor arteries in patients with colorectal cancer. Rev Esp Enferm Dig 2008; 100: 327-31.
12. Kramer HJ, Schwarting K, Baker A, Meyer-Lehnert H. Renal endothelin system in obstructive jaundice: its role in impaired renal function of bile-duct ligated rats. Clin Sci (Lond) 1997; 92: 579-85.
13. Sarac AM, Aktan AÖ, Mohín FH, Bilsel S, Scapa E. Role of endothelin in obstructive jaundice. Dig Dis Sci 1999; 44: 356-63.
14. Oms L, Martínez-Ródenas F, Valverde J, Jiménez W, Sitges-Serra A. Reduced water and sodium intakes associated with high levels of natriuretic factor following common bile ligation in the rabbit. Br J Surg 1990; 77: 752-5.
15. Gallardo JM, Padillo J, Martín-Malo A, Miño G, Pera C, Sitges-Serra A. Increased plasma levels of atrial natriuretic peptide and endocrine markers of volume depletion in patients with obstructive jaundice. Br J Surg 1998; 85: 28-31.
16. Padillo FJ, Rodriguez M, Gallardo JM, Andicoberry B, Naranjo A, Martin-Malo A, et al. Preoperative assessment of body fluid disturbances in patients with obstructive jaundice. World J Surg 1999; 23: 681-7.
17. Kojima H, Sakurai S, Kuriyama S, Yoshiji H, Imazu H, Uemura M, et al. Endothelin-1 plays a major role in portal hypertension of biliary cirrhotic rats through endothelin receptor subtype B together with subtype A in vivo. J Hepatol 2001; 34: 805-11.
18. Ling Y, Zhang J, Luo B, Song D, Liu L, Tang L, et al. The role of endothelin-1 and the endothelin B receptor in the pathogenesis of hepatopulmonary syndrome in the rat. Hepatology 2004; 39: 1593-602.
19. Palmes D, Skawran S, Stratmann U, Arman B, Minin E, Herbst H, Spiegel HU. Amelioration of microcirculatory damage by an endothelin receptor antagonist in a rat model of reversible acute liver failure. J Hepatol 2005; 42: 350-7.
20. Tièche S, De Gottardi A, Kappeler A, Shaw S, Sägesser H, Zimmermann A, et al. Overexpression of endothelin-1 in bile duct ligated rats: correlation with activation of hepatic stellate cells and portal pressure. J Hepatol 2001; 34: 38-45.
21. Luo B, Liu L, Tang L, Zhang J, Ling Y, Fallon MB. ET-1 and TNF-α in HPS: analysis in prehepatic portal hypertension and biliary and nonbiliary cirrhosis in rats. Am J Physiol Gstrointest Liver Physiol 2004; 286: G294-G303.
22. Bark AM, Abadía AF, El-Marsafawy, Abu-Hashem I, El-Regal ME, Amer T, et al. Plasma endothelin-1 concentrations in children with cirrhosis and their relationship to renal function and the severity of portal hypertension. J Pediatr Gastroenterol Nutr 2002; 2: 149-53.
23. Kohan DE. Endothelins in the kidney. Physiology and pathophysiology. Am J Kidney Dis 1993; 33: 493-510.
24. O'Neill PA, Wait RB, Kahng KU. Role of renal sympathetic nerve activity in renal failure associated with obstructive jaundice in the rat. Am J Surg 1991; 161: 662-7.
25. Uemura M, Tsujii T, Fukui H, Takaya K, Tsukamoto N, Nakayama M, et al. Urinary prostaglandins and renal function in jaundice obstructive. Scand J Gastroenterol 1989; 24: 705-15.